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FACTORS RELATED TO FECAL ESTROGENS AND FECAL TESTOSTERONE IN CALIFORNIA SPOTTED OWLS

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Abstract. We estimated concentrations of fecal reproductive steroid metabolites in free-ranging California Spotted Owls (*Strix occidentalis occidentalis*) during the breeding season. We collected fresh fecal samples ($n = 142$) from 65 individual owls in the Sierra Nevada during April–August of 2001. We developed and validated radioimmunoassay procedures to quantify fecal estrogen metabolites and fecal testosterone metabolites. We used an information-theoretic approach to identify factors that might influence fecal estrogen (E), fecal testosterone (T), and fecal estrogen:testosterone ratio (E:T ratio) levels during the owl's breeding season. We hypothesized that factors related to sampling procedures, owl characteristics (sex, reproductive status), and habitat might influence fecal reproductive steroid levels. Our analyses suggested that sampling factors and owl characteristics, but not habitat variables, were related to fecal reproductive steroid levels in Spotted Owls. Our most supported models explained <30% of the observed variation. Fecal testosterone levels were higher in male Spotted Owls than females, whereas E:T ratios were higher in females compared to males. High fecal estrogens were correlated with high fecal glucocorticoids in nonbreeding Spotted Owls, whereas fecal estrogens and fecal glucocorticoids were not related in breeding birds. Sampling influenced fecal reproductive steroid measures, and bias from small-mass fecal samples might partially explain these relationships. Noninvasive measurements of fecal reproductive steroids might be useful for sex determination and reproductive assessment of free-ranging Spotted Owls. However, more research is needed to understand the variability we observed in sex steroids before this technique can be effective in conservation studies.

Key words: California Spotted Owl, estrogens, glucocorticoids, noninvasive, testosterone, physiology, *Strix occidentalis occidentalis*.

Factores Asociados con los Estrógenos Fecales y la Testosterona Fecal en *Strix occidentalis occidentalis*

Resumen. Estimamos las concentraciones de metabolitos esteroides reproductivos en individuos silvestres de la especie *Strix occidentalis occidentalis* durante la época reproductiva. Colectamos muestras fecales frescas ($n = 142$) pertenecientes a 65 lechuzas en la Sierra Nevada entre abril y agosto de 2001. Desarrollamos y validamos un procedimiento de radioinmunoensayo para cuantificar metabolitos de estrógeno fecales y metabolitos de testosterona fecales. Utilizamos un enfoque informativo-teórico para identificar los factores que podrían influenciar los niveles de estrógenos fecales (E), testosterona fecal (T) y el cociente entre estrógenos y testosterona (cociente E:T) durante la época reproductiva de las lechuzas. Hipotetizamos que factores relacionados con los procedimientos de muestreo, características de la lechuza (sexo, estado reproductivo) y el hábitat podrían influenciar los niveles de esteroides reproductivos en las fecas. Nuestros análisis sugieren que los factores asociados al muestreo y las características de la lechuza se correlacionaron con los niveles de esteroides reproductivos en las fecas, pero éstos no se relacionaron con las variables de hábitat. Nuestro modelo más robusto explicó <30% de la variación observada. Los niveles de testosterona fecal fueron mayores en los machos que en las hembras, mientras que el cociente E:T fue mayor en las hembras que en los machos. En lechuzas no reproductivas, los niveles altos de estrógenos fecales se correlacionaron con niveles altos de glucocorticoides fecales, mientras que en individuos reproductivos los estrógenos fecales y los glu-

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corticoides fecales no se correlacionaron. Estas relaciones pueden ser explicadas en parte por la influencia del muestreo sobre las medidas de esteroides reproductivos fecales y por el sesgo causado por muestras fecales muy livianas. Los niveles de esteroides reproductivos fecales medidos con técnicas no invasivas pueden ser útiles para la determinación de sexos y la evaluación del estado reproductivo de individuos silvestres de la especie *S. occidentalis occidentalis*. Sin embargo, antes de que esta técnica pueda ser efectiva en estudios de conservación, se requiere de más investigación para entender la variabilidad que observamos en los niveles de esteroides sexuales.

INTRODUCTION

The Northern and Mexican Spotted Owls (*Strix occidentalis caurina* and *S. o. lucida*) are listed in the United States as threatened subspecies, whereas the California subspecies (*S. o. occidentalis*) is not (USDI 1990, 1993, 2003). Because the conservation status of Spotted Owls has great economic ramifications, there has been extensive research on its biology (Gutiérrez et al. 1995, Franklin et al. 2004). The biological status of the California Spotted Owl is of interest because forest management practices and habitat conditions within its range are diverse (Verner et al. 1992). In particular, Sierra Nevada forests, where most California Spotted Owls reside, have been managed using a wide variety of silvicultural practices, with much less emphasis on clearcutting than in the range of the Northern Spotted Owl. Also, the California Spotted Owl is generally well distributed within its limited range (Verner et al. 1992).

Central to current Spotted Owl research has been a desire to understand the mechanisms that influence its population dynamics and habitat selection (Franklin et al. 2000, 2004). Because experiments with this species are difficult to execute (Noon and Franklin 2002), alternative approaches are being used to elucidate basic ecological relationships such as population meta-analysis (Burnham et al. 1996), population genetics (Barrowclough et al. 1999), and the effects of disturbance using noninvasive techniques (Wasser et al. 1997, Tempel and Gutiérrez 2004). A field protocol exists for assessing reproductive status in owls (Franklin et al. 1996), but a negative assessment using this protocol does not always mean that the bird did not attempt to nest. One noninvasive technique that might provide useful insight into the reproductive biology of the Spotted Owl is the evaluation of fecal reproductive hormone levels (i.e., testosterone and estrogen) as a measure of physiological response to social and environmental conditions.

Reproductive steroid hormone levels provide important information regarding the reproductive status of animals. However, longitudinal studies of reproductive function in free-ranging Spotted Owls using traditional techniques (i.e., blood sampling) would require repeatedly subjecting owls to the stress of capture, restraint, and blood collection (Le Maho et al. 1992). In contrast, noninvasive monitoring of reproductive steroids using fecal hormone metabolites is advantageous because sample collection does not require animal capture, samples can be collected without disturbing study animals, and numerous samples can be collected from individuals (Bercovitz et al. 1982, Kofuji et al. 1993). Our objectives were: (1) to validate radioimmunoassay (RIA) procedures to quantify estrogen and testosterone metabolites in Spotted Owl feces and (2) to evaluate factors that might explain variation in fecal estrogen (E), fecal testosterone (T), and estrogen:testosterone ratio (E:T ratio) levels in free-ranging California Spotted Owls during the breeding season.

METHODS

STUDY AREA

Our 925-km² study area was located in the north-central Sierra Nevada between Georgetown and South Lake Tahoe, California (39°N, 120°W). During 1986–2001, we surveyed a core area of 355 km² each year for all Spotted Owls plus selected territories within an adjacent 570 km² (Seamans et al. 2001). Public (USDA Forest Service) and private land comprised 63% and 37% of the core area, respectively.

Elevation on the study area ranged from 366 m to 2257 m. Vegetation was typical Sierran mixed-conifer forest (Küchler 1977, Rundel et al. 1977). From 600 m to 1500 m the forests were dominated by ponderosa pine (*Pinus ponderosa*) on more xeric sites and white fir (*Abies concolor*) on more mesic sites. Above 1500 m there was a transition zone dominated by red fir (*Abies magnifica*). Other common tree species

that occurred within the study area included sugar pine (*Pinus lambertiana*), Douglas-fir (*Pseudotsuga menziesii*), incense cedar (*Calocedrus decurrens*), canyon live oak (*Quercus chrysolepis*), California black oak (*Quercus kelloggii*), and Pacific dogwood (*Cornus nuttallii*).

FECAL SAMPLE COLLECTION

We collected fecal samples from April to August 2001, with early April marking the onset of Spotted Owl nesting on the study area. We located owls using standard techniques (Forsman 1983). We placed a clean polyethylene sheet beneath a roosting owl (when possible) to facilitate sample collection. We removed the fecal portion of excreta from the urine portion (Wasser et al. 1997) and placed the fecal samples into cryogenic vials. Fecal samples were then placed into liquid nitrogen or placed on ice until they could be transferred to liquid nitrogen. We collected fecal samples from adults and did not use cecal discharge samples (Tempel and Gutiérrez 2004). Most individual owls on the study area were color banded, and unmarked birds were captured and color banded at the first opportunity. We used these color bands to assign fecal samples to individuals. The sex of all birds was determined by vocal characteristics or nesting behavior (Forsman 1983, Franklin et al. 1996).

FECAL SAMPLE PREPARATION

We placed frozen fecal samples in a lyophilizer (Freeze-dry Specialties, Inc., Osseo, Minnesota) for 24 hr. Once samples were freeze-dried, we ground them, sifted them through a stainless steel mesh to remove large particles, and mixed them thoroughly. We weighed each fecal sample to the nearest 0.001 g. We extracted glucocorticoids from feces using a modification of Schwarzenberger et al. (1991). We placed dried feces (~0.1 g) in a test tube with 2.0 mL of 90% methanol and vortexed at high speed in a multitube vortexer for 30 min. Samples were then centrifuged at 500 g for 20 min, and the supernatant was saved and stored at -84°C.

RADIOIMMUNOASSAY PROCEDURES AND ASSAY VALIDATION

We quantified estrogen metabolite levels in Spotted Owl fecal extracts using a commercially available total estrogens I¹²⁵ double-antibody radioimmunoassay kit (Cat. #07-140202, ICN Biomedicals, Costa Mesa, California). We fol-

lowed the ICN protocol for the total estrogens I¹²⁵ RIA. We measured testosterone metabolites in Spotted Owl feces using a commercially available testosterone I¹²⁵ double-antibody radioimmunoassay kit (Cat. #DSL-4100, Diagnostic Systems Laboratories, Webster, Texas). We followed the manufacturer's method for the testosterone I¹²⁵ RIA, except that we halved the volume of all reagents. We conducted standard validations including parallelism and recovery of exogenous sex steroid validation assays (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000) on six pooled fecal extracts to confirm these assays were accurately and precisely measuring estrogen and testosterone metabolites in Spotted Owl feces. Pooled fecal extracts (expected low, medium, and high levels) were composed of fecal extracts from three males and three females. We added exogenous testosterone to the medium and high pool fecal extracts to obtain testosterone values under higher dilution levels. Interassay variation was calculated from the three owl samples. Intra-assay variation was calculated by averaging the CVs of replicate tubes from 20 randomly chosen samples. We used tests for equal slopes (parallelism) to determine if log-transformed curves of serially diluted pool fecal extracts were parallel to log-transformed total estrogen and testosterone standard curves (Neter et al. 1990). We used SAS (SAS Institute 1985) to perform all statistical analyses.

FACTORS RELATED TO REPRODUCTIVE STEROID LEVELS

Based on the literature, we developed *a priori* verbal hypotheses regarding the correlation of various factors with fecal estrogen, fecal testosterone, and estrogen:testosterone (E:T) ratio levels in California Spotted Owls during the breeding season. First, we hypothesized that sample collection or storage factors might influence fecal reproductive steroid concentrations (sampling hypothesis). Second, we hypothesized that characteristics of individual owls and the timing of sample collection might influence fecal reproductive steroid levels (biological hypothesis). Last, we hypothesized that habitat characteristics might be related to fecal reproductive steroid levels (habitat hypothesis). For each hypothesis, we developed a set of *a priori* models. We used several covariates to develop the set of *a priori* candidate models representing multiple hypoth-

eses of covariate effects on estrogen metabolites, testosterone metabolites, and E:T ratios in owl feces (Appendix A; Burnham and Anderson 1998).

Sampling covariates. Sampling covariates included the initial storage method for collected samples (SSTOR) and the mass of an individual fecal sample (SMASS). Storage methods included placing vials containing feces into liquid nitrogen within 6 hr of collection or storage of vials on ice for up to 36 hr before storage in liquid nitrogen. Sample mass was the mass of the fecal sample (to the nearest 0.001 g) after freeze-drying and sieving. Tempel and Gutiérrez (2004) found that fecal glucocorticoids were higher in Spotted Owl feces initially stored on ice and in samples of small mass. Thus, we predicted fecal estrogens and fecal testosterone would be higher in samples initially stored on ice and samples having smaller mass (Appendix B).

Biological covariates. Biological covariates included sex of the bird (SEX), reproductive life-history stage (RLHS) of the bird, fecal glucocorticoid level (CORT), and sample collection date (DATE). We determined the sex of an individual owl from its unique color band or by the pitch of its call (Forsman et al. 1984). We hypothesized that male Spotted Owls would have higher fecal testosterone levels than females, whereas females would have higher fecal estrogen and E:T ratios than males (Appendix B). We estimated the reproductive life-history stage of each individual owl using the methods described in Franklin et al. (1996). Each individual owl was classified as nonbreeding or breeding (i.e., actively nesting or fledging young). We expected breeding owls to have higher fecal reproductive steroid levels than nonbreeding owls. Glucocorticoid metabolite concentrations were estimated as described in Tempel and Gutiérrez (2004). Since elevated corticosterone levels might suppress reproduction in birds (Wingfield 1988, Wingfield et al. 1998), we hypothesized that owls with high fecal glucocorticoid levels would have lower reproductive steroid levels.

Habitat covariates. Habitat covariates included the amount of core area in the owl's territory (CORE) and the number of discrete habitat patches in the owl's territory (PATCH). We defined an owl territory as a circle with a radius of one-half the mean nearest-neighbor distance

between territory centers (Peery et al. 1999, Franklin et al. 2000). A territory center was the location of a nest site, or for non-nesting owls, the average location of roost sites recorded at least 1 week apart. Most roost sites within a territory were near each other within the same forest stand. Mean nearest-neighbor distance was estimated for the year of highest owl density on the study area (1996).

We defined owl habitat as all mature conifer forest, irrespective of canopy cover, and medium-sized conifer forest with canopy cover $\geq 70\%$ because California Spotted Owls nest, roost, and forage in these habitats (Gutiérrez et al. 1992). Mature forest consisted of stands having dominant trees ≥ 61 cm diameter at breast height (dbh), and mid-seral forest had dominant trees ranging from 30 to 60 cm dbh. We estimated habitat variables as described in Tempel and Gutiérrez (2004).

Franklin et al. (2000) found that territory-level habitat characteristics (e.g., the amount of core owl habitat within a Spotted Owl's territory) were important predictors of reproductive output in Northern Spotted Owls. Thus, we hypothesized that the amount of core area in the owl's territory or the number of discrete habitat patches in the owl's territory influenced reproductive steroid levels in California Spotted Owls. Specifically, we predicted fecal estrogen and fecal testosterone concentrations would be higher in owls having more core area and fewer habitat patches in their territory (Appendix B).

Model selection. We used information-theoretic model selection (Burnham and Anderson 1998) using linear mixed models (PROC MIXED in SAS) where individual owls were the sampling unit, the date of fecal sample collection was a repeated effect, and all other covariates were fixed effects (Littell et al. 1996). Potential nonindependence among fecal samples from the same individual was accounted for by the repeated-measures analysis. To account for possible heterogeneous sampling variances among individuals, we first used restricted maximum-likelihood estimation to select the appropriate covariance structure for the global model using PROC MIXED (Littell et al. 1996); the sample variance structures were ranked using AIC_c (a small-sample-size correction for Akaike's Information Criterion). The diagonal matrix grouped by sex was the best fit for estrogens and

TABLE 1. Likelihood-ratio tests for goodness-of-fit of subglobal models estimating fecal estrogens, fecal testosterone, and fecal estrogens:testosterone ratios in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001. Explanations of covariate abbreviations appear in Appendix A. *A priori* models that contained covariates from subglobal models that were not significant were not included in the final model-selection subset.

Subglobal model	Estrogens		Testosterone		E:T ratio	
	χ^2_2	<i>P</i>	χ^2_2	<i>P</i>	χ^2_2	<i>P</i>
Sampling ^a	10.2	0.006	30.8	<0.001	9.5	0.009
SEX*RLHS ^b	17.7	<0.001	14.9	<0.001	36.3	<0.001
CORT*RLHS ^c	40.4	<0.001	19.8	<0.001	5.4	0.07
CORT*DATE ^d	31.8	<0.001	21.4	<0.001	1.4	0.50
Habitat ^e	0.4	0.82	3.3	0.19	4.5	0.82

^a The Sampling subglobal model contained SMASS, SSTOR, and SMASS*SSTOR.

^b The SEX*RLHS subglobal model contained SEX, RLHS, and SEX*RLHS.

^c The CORT*RLHS subglobal model contained CORT, RLHS, and CORT*RLHS.

^d The CORT*DATE subglobal model contained CORT, DATE, (DATE)², and CORT*DATE.

^e The habitat subglobal model contained CORE, PATCH, and CORE*PATCH.

testosterone, whereas the Gaussian spatial structure grouped by sex was the best fit for E:T.

We used a two-stage approach to model fitting (Franklin et al. 2000, Seamans et al. 2001). First, we compared models containing only sampling covariates. We used AIC_c to select the sampling model with the most support for each of the three hormone response variables. Second, we compared models containing biological and habitat covariates. To each of the *a priori* biological and habitat models, we also included the covariate(s) from the best-fit sampling model. We assessed goodness-of-fit using likelihood-ratio tests for a series of subglobal models and removed those models which did not have significant fit from consideration (Table 1). We considered test results significant for *P* < 0.05. For the final evaluation, *a priori* models were ranked using AIC_c (Burnham and Anderson 1998).

RESULTS

VALIDATION OF RADIOIMMUNOASSAY

Fecal estrogens. The I¹²⁵ RIA reliably quantified estrogen metabolites in Spotted Owl feces. Serial dilutions (1:2 up to 1:128) of low, medium, and high pool fecal extracts yielded displacement curves parallel to the estrogens standard curve (Fig. 1; all *P* > 0.2). Mean recovery of exogenous total estrogens (range 10–50 pg mL⁻¹) added to low and high pool fecal extracts was 108.2 ± 1.6% (*n* = 18). Acceptable recovery of exogenous analyte (within 90–110%) and demonstration of parallelism suggested no sample matrix effects (Jeffcoate 1981, Grotjan and Keel 1996, O’Fegan 2000). Extracts from fecal

samples were diluted with steroid diluent to 1:64 (e.g., 50 µL of fecal extract into 1.55 mL of steroid diluent) prior to assay. Intra-assay variation was 2.4%, calculated from 20 randomly chosen samples, and interassay variation for five assays was 11.2%. The manufacturer’s reported cross-reactivity of ICN total estrogens antisera was 100% with estrone and estradiol 17-β, 9% with estriol, 7% with estradiol 17-α, and <1% for other steroids. Assay sensitivity was 100 pg g⁻¹.

Fecal testosterone. The DSL testosterone I¹²⁵ RIA reliably quantified testosterone metabolites in Spotted Owl feces. Serial dilutions (1:2 up to 1:128) of low, medium, and high pool fecal extracts yielded displacement curves that were parallel to the testosterone standard curve (Fig. 1; all *P* > 0.3). Mean recovery of exogenous testosterone (range 0.5–10 ng mL⁻¹; levels chosen to correspond with expected fecal testosterone levels from actual samples) added to low and high pool fecal extracts was 94.1 ± 2.9% (*n* = 18). Acceptable recovery of exogenous analyte (within 90–110%) and demonstration of parallelism suggested no sample matrix effects (Jeffcoate 1981, Grotjan and Keel 1996, O’Fegan 2000). Extracts from fecal samples were not diluted in assay diluent prior to assay. Intra-assay variation, calculated from 20 randomly chosen samples, was 2.2%. Interassay variation for the five assays was 6.7%. The manufacturer’s reported cross-reactivity of DSL testosterone antisera was 100% with testosterone, 6.6% with 5α-dihydrotestosterone, 2.2% with 5-androstane-3β, 17β-diol, 1.8% with 11-oxotestoste-

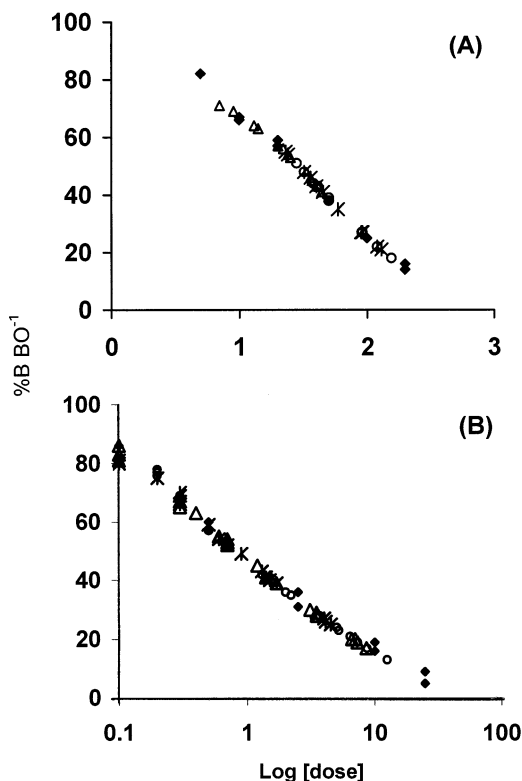


FIGURE 1. Parallelism results for (A) fecal estrogens and (B) fecal testosterone for California Spotted Owls. Curves of percent binding of I^{125} tracer ($\%B\ Bo^{-1}$) versus serially diluted (log-transformed doses of 1:2 up to 1:128) low pool ($n = 2$), medium pool ($n = 2$), and high pool ($n = 2$) fecal extracts from male and female free-ranging California Spotted Owls were parallel (test of equal slopes, all $P > 0.3$) to total estrogen (log-transformed doses of 5–200 $pg\ mL^{-1}$) or testosterone standard curves (log-transformed doses of 0.1–25 $ng\ mL^{-1}$). Diamonds: total estrogen or testosterone standard curve points, circles: serially diluted low fecal extracts, triangles: serially diluted medium pool fecal extracts, and stars: serially diluted high pool fecal extracts.

rone, and $<1\%$ for other steroids. The sensitivity of this assay was 2 $ng\ g^{-1}$.

CORRELATES OF REPRODUCTIVE STEROID LEVELS

During the 2001 breeding season, we collected and assayed 142 fecal samples from 65 individual California Spotted Owls (32 females and 33 males) for fecal estrogen and fecal testosterone metabolites. We collected an average of 2.2 ± 0.1 (range 1–6) fecal samples per individual owl.

For each of the three response variables (estrogen, testosterone, E:T), the sampling subglobal model adequately fit the data (Table 1). Among the four sampling models, the model $ESTR_{SMASS+SSTOR}$ best fit the estrogens data and thus was included with second-stage models (Table 2). The model $TEST_{SMASS}$ best fit the testosterone data and thus was included with second-stage models (Table 3). Similarly, for the E:T data, the model $E:T_{SMASS}$ was the best supported and was included with second-stage models (Table 4).

For all three response variables, the $SEX*RLHS$ subglobal model adequately fit the data, whereas the habitat subglobal model did not (Table 1). The $CORT*RLHS$ and $CORT*DATE$ subglobal models adequately fit the estrogens and testosterone data, but did not fit the E:T data (Table 1).

For fecal estrogens, the model $ESTR_{CORT*RLHS}$ was the best supported, with more than double the AIC_c weight of the nearest competing model (Table 2). The $ESTR_{CORT*RLHS}$ model explained 25% of the total observed variation. The nearest competing model, $ESTR_{CORT*RLHS+SMASS+SSTOR}$, was also well supported and was within two ΔAIC_c units of the best model. Both models indicated that fecal estrogen levels were positively related to fecal glucocorticoid levels in non-breeding Spotted Owls and unrelated to fecal glucocorticoids in breeding owls. The second model indicated that fecal estrogen levels were negatively related to sample mass and initial storage of the samples in liquid nitrogen.

Among the fecal testosterone models, the model $TEST_{SEX+SMASS}$ had the lowest AIC_c value, and its AIC_c weight was more than three times higher than the other models (Table 3). This model explained 29% of the observed variation. The nearest competing models were $TEST_{SEX+RLHS+SMASS}$ and $TEST_{SEX*RLHS+SMASS}$, which were also supported and within four ΔAIC_c units of the best model. These three models indicated that male California Spotted Owls had higher fecal testosterone levels than females and that fecal testosterone levels decreased as the mass of fecal samples increased. Additionally, two of the models suggested that breeding owls had higher fecal testosterone levels.

For E:T, the most supported models were $E:T_{SEX*RLHS+SMASS}$, which explained 28% of the total observed variation, and $E:T_{SEX+RLHS+SMASS}$, which explained 27% of the total observed var-

TABLE 2. Ranking of *a priori* models estimating fecal estrogens in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001. Models were ranked based on Akaike's Information Criterion adjusted for small sample size (AIC_c). ΔAIC_c is the difference in AIC_c between a model and the best-approximating model. AIC_c weights sum to 1 and indicate the relative likelihood of the current model. See appendices for explanation of covariate abbreviations and model hypotheses.

Estrogens model	$\log (l)^a$	k^b	ΔAIC_c^c	AIC_c weight
Sampling				
SMASS + SSTOR ^d	–557.2	5	0.0	0.53
Overall				
CORT*RLHS	–542.1	6	0.0	0.63
CORT*RLHS + SMASS + SSTOR	–540.3	8	1.6	0.28
CORT + SMASS + SSTOR	–545.3	6	6.5	0.02
DATE + CORT	–546.6	5	6.7	0.02
CORT	–547.9	4	6.9	0.02
DATE + CORT + SMASS + SSTOR	–544.8	7	8.0	0.01
DATE*CORT	–546.4	6	8.6	0.01
DATE*CORT + SMASS + SSTOR	–544.5	8	10.0	0.00

^a Maximized log-likelihood value.

^b Number of model parameters.

^c The lowest AIC_c score was 1125.4 for the sampling models and 1097.5 for the overall models.

^d Covariates from this model were then included in the overall models.

iation. Both E:T models indicated that E:T ratios were higher in female California Spotted Owls (Fig. 2). The nearest competing model was E: T_{SEX+SMASS}, which was also well supported and within two ΔAIC_c units of the best model (Table 4). All three models indicated that the ratio of E:T was higher in female Spotted Owls compared to males (Fig. 2) and that E:T ratios were negatively related to sample mass. Additionally, two of the models suggested the reproductive

life-history stage of individual owls was correlated with E:T (Fig. 2).

DISCUSSION

Our findings indicated the ICN I¹²⁵ double-antibody total estrogens RIA and the DSL I¹²⁵ double-antibody testosterone RIA used in this study were effective for quantifying immunoreactive estrogen and testosterone metabolites, respectively, in feces from male and female Spotted

TABLE 3. Ranking of *a priori* models estimating fecal testosterone in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001. Models were ranked based on Akaike's Information Criterion adjusted for small sample size (AIC_c). ΔAIC_c is the difference in AIC_c between a model and the best-approximating model. AIC_c weights sum to 1 and indicate the relative likelihood of the current model. See appendices for explanation of covariate abbreviations and model hypotheses.

Testosterone model	$\log (l)^a$	k^b	ΔAIC_c^c	AIC_c weight
Sampling				
SMASS ^d	–481.3	4	0.0	0.53
Overall				
SEX + SMASS	–471.8	5	0.0	0.68
SEX + RLHS + SMASS	–471.8	6	2.4	0.20
SEX*RLHS + SMASS	–471.1	7	3.5	0.12
CORT + SMASS	–476.8	5	10.0	0.01
DATE + CORT + SMASS	–476.6	6	12.1	0.00

^a Maximized log-likelihood value.

^b Number of model parameters.

^c The lowest AIC_c score was 971.3 for the sampling models and 954.6 for the overall models.

^d Covariates from this model were then included in the overall models.

TABLE 4. Ranking of *a priori* models estimating fecal estrogen:testosterone ratios (E:T) in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001. Models were ranked based on Akaike's Information Criterion adjusted for small sample size (AIC_c). ΔAIC_c is the difference in AIC_c between a model and the best-approximating model. AIC_c weights sum to 1 and indicate the relative likelihood of the current model. See appendices for explanation of covariate abbreviations and model hypotheses.

E:T ratio model	$\log (l)^a$	k^b	ΔAIC_c^c	AIC_c weight
Sampling				
SMASS ^d	-273.1	6	0.0	0.53
Overall				
SEX*RLHS + SMASS	-254.2	9	0.0	0.39
SEX + RLHS + SMASS	-255.6	8	0.1	0.37
SEX + SMASS	-257.4	7	1.1	0.23
SEX*RLHS	-259.7	8	8.2	0.01
SEX + RLHS	-261.0	7	8.3	0.01
SEX	-262.9	6	9.6	0.00

^a Maximized log-likelihood value.

^b Number of model parameters.

^c The lowest AIC_c score was 559.6 for the sampling models and 529.7 for the overall models.

^d Covariates from this model were then included in the overall models.

Owls. The performance characteristics (e.g., parallelism, recovery of exogenous analyte, accuracy, precision) of these assays verified that they were accurate, precise, and had an appropriate range of sensitivity. Monfort et al. (1997) successfully used the same total estrogens and testosterone assays to quantify fecal estrogen and testosterone metabolites, respectively, in African wild dogs (*Lycaon pictus*). Our findings corroborate other studies suggesting the reproductive condition of captive (Bishop and Hall 1991, Bercovitz et al. 1982, Lee et al. 1995) and free-ranging (Kofuji et al. 1993, Cockrem and Rounce 1995) birds can be monitored using fecal reproductive steroid metabolite assays. Additionally, studies of domestic geese (*Anser anser*; Hirschenhauser et al. 2000) and domestic fowl (*Gallus domesticus*; Cockrem and Rounce 1994) have demonstrated that fecal levels of reproductive steroid metabolites were related to plasma reproductive steroids.

Previous studies have demonstrated that fecal reproductive steroid analyses can be used to determine the sex of individuals from monomorphic bird species (Bercovitz et al. 1978, Stavy et al. 1979). The E:T ratio has been shown to be particularly useful. Our findings suggest E:T ratios from California Spotted Owl feces might be used to determine the sex of the owl. An E:T ratio of 2.9 or higher indicated the owl was a female, whereas an E:T ratio of 1.6 or less indicated the owl was a male. Further studies us-

ing fecal samples from Spotted Owls of known sex will be needed to verify the usefulness of this technique and to determine the potential error rate for sex identification.

Sampling factors and characteristics of individual owls influenced reproductive steroid metabolite levels in California Spotted Owl feces during the breeding season. The amount of feces available for analysis influenced the estrogen and testosterone measurements in Spotted Owls. Consequently, the E:T ratios also were influenced by fecal sample mass. Tempel and Gutiérrez (2004) found a similar relationship between fecal mass and fecal glucocorticoid levels in California Spotted Owls. Samples of small mass (e.g., 0.02 g dry weight) might have been biased high. Although the reason for this bias was unknown, we suspected the extraction efficiency of steroid metabolites from the fecal material may have been higher with very small fecal samples. Researchers should be aware that small samples might provide spurious results. Thus, we suggest that in future studies of fecal reproductive steroids in Spotted Owls, researchers decide *a priori* whether to exclude very small (e.g., <0.02 g dry weight) fecal samples from analysis. Additionally, the uric acid portion of the samples, which might vary among samples, could have influenced our results.

Fecal samples with high levels of fecal estrogen metabolites also had high levels of fecal glucocorticoids. This finding was unexpected, as

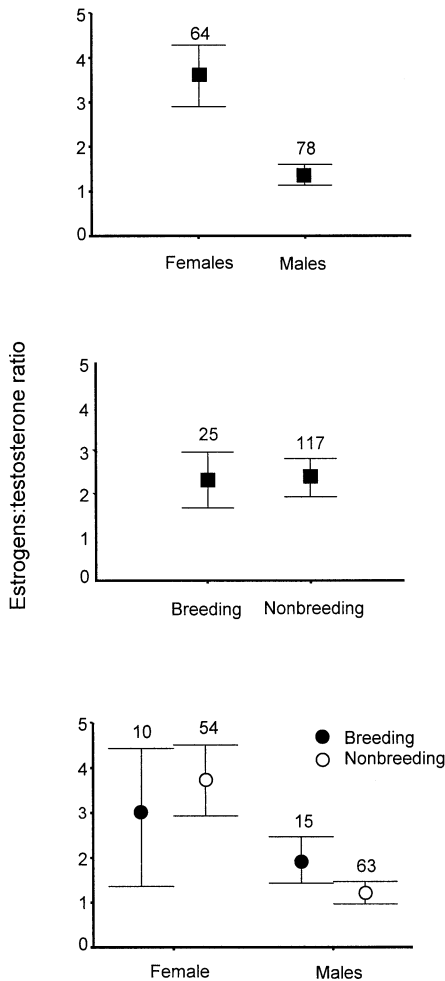


FIGURE 2. Estrogen:testosterone ratios of California Spotted Owls in the central Sierra Nevada, California, during April–August 2001. Bars represent 95% CI. (A) adult males and females, (B) breeding and nonbreeding owls, and (C) male and female breeding (filled circles) and nonbreeding (unfilled circles) owls.

chronically high corticosterone levels have been shown to suppress reproduction in birds (Wingfield 1988, Wingfield et al. 1998). Sampling factors (e.g., sample mass) also influence fecal glucocorticoid and fecal reproductive steroid concentrations (Tempel and Gutiérrez 2004; this study). We suspected one or more factors that elevated fecal glucocorticoid levels also elevated fecal estrogen levels in the same individuals. That is, birds in breeding status might have a concomitant increase in stress hormone secretion related to permissive actions of glucocorti-

coids (such as metabolizing energy reserves) unrelated to stress responses.

While we observed that variability in sex steroids was related to sampling and sex of the birds, our models explained relatively little variation. Our results suggested a more complex relationship among Spotted Owl sex steroids and features of the environment than could be explained by our *a priori* models. As with other fecal steroid techniques (e.g., glucocorticoid assays), it will be important to understand the biotic and abiotic factors influencing these hormones before the real utility of these techniques can be realized. Basic studies that examine individual variation and between-year variation in reproductive steroids among birds in different physical conditions and life-history stages will be needed to understand variability in fecal reproductive steroid metabolites. Further work is needed to determine the influence of fecal sample collection and processing on fecal reproductive steroid measurements. In addition, the interrelationships between acute and chronic stress responses and reproductive hormone levels need to be thoroughly examined. These issues will necessitate the study of birds under controlled conditions where environmental conditions (e.g., food availability) and sample handling are altered.

In summary, we validated radioimmunoassay procedures that quantify estrogen and testosterone metabolites in Spotted Owl feces. Noninvasive monitoring of reproductive steroid metabolite levels in feces, used in combination with demographic information (e.g., reproductive indices), may provide an effective tool for examining the effects of various naturally occurring and anthropogenic influences on the reproductive aspects of Spotted Owl populations. Future research examining how fecal reproductive steroid levels may vary between male and female Spotted Owls during early stages of the breeding cycle (e.g., courtship), during the nonbreeding season, in juvenile birds, and in other owl populations inhabiting areas of differing habitat quality are necessary. Additional research is needed to assess fundamental issues related to fecal reproductive steroid metabolites, such as sampling issues and understanding normal profiles in birds of various conditions and life history stages, before the technique can be applied most effectively.

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APPENDIX A. Covariates used in *a priori* models to estimate fecal estrogens, fecal testosterone, and fecal estrogens:testosterone ratios in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001.

Variable	Description
Sampling	
SSTOR	Storage method (same initially stored in liquid N ₂ or on ice)
SMASS	Mass of dried sample analyzed (to the nearest 0.001 g)
Biological	
SEX	Gender of the bird (female or male)
RLHS	Reproductive life-history stage (breeding or nonbreeding)
CORT	Fecal glucocorticoid metabolite level (ng g ⁻¹) estimated from the same sample
DATE	Day of year (Julian date)
Habitat	
CORE	Amount of interior Spotted Owl habitat (>100 m from an edge) within the owl's territory (km ²)
PATCH	Number of discrete patches of Spotted Owl habitat within the owl's territory

APPENDIX B. Description, representation, and predicted directions of *a priori* models to estimate fecal estrogens, fecal testosterone, and fecal estrogens:testosterone ratios in California Spotted Owls in the north-central Sierra Nevada, California, April–August 2001. Each model was evaluated for each of the three response variables (estrogens, testosterone, and E:T).

Hypothesis	Model	Model structure	Predicted effects ^a
Sampling			
Differences are due to sample mass	SMASS	$\beta_0 + \beta_1(\text{SMASS})$	$\beta_{\text{SMASS}} < 0$
Differences are due to sample storage method	SSTOR	$\beta_0 + \beta_1(\text{SSTOR})$	$\beta_{\text{SSTOR}} < 0$
Differences are due to both sample mass and sample storage method	SMASS + SSTOR	$\beta_0 + \beta_1(\text{SMASS}) + \beta_2(\text{SSTOR})$	$\beta_{\text{SMASS}} < 0, \beta_{\text{SSTOR}} < 0$
Sample mass-specific differences are related to sample storage method	SMASS*SSTOR	$\beta_0 + \beta_1(\text{SMASS}) + \beta_2(\text{SSTOR}) + \beta_3(\text{SMASS*SSTOR})$	$\beta_{\text{SMASS}} > 0, \beta_{\text{SSTOR}} < 0, \beta_{\text{SMASS*SSTOR}} < 0$
Biological			
Differences are due to sex of the bird	SEX	$\beta_0 + \beta_1(\text{SEX})$	$\beta_{\text{SEX}} > 0$
Differences are due to reproductive life-history stage of the bird	RLHS	$\beta_0 + \beta_1(\text{RLHS})$	$\beta_{\text{RLHS}} > 0$
Differences are due to sex of the bird and reproductive life-history stage of the bird	SEX + RLHS	$\beta_0 + \beta_1(\text{SEX}) + \beta_2(\text{RLHS})$	$\beta_{\text{SEX}} > 0, \beta_{\text{RLHS}} > 0$
Sex-specific differences are related to the reproductive life-history stage of the bird	SEX*RLHS	$\beta_0 + \beta_1(\text{SEX}) + \beta_2(\text{RLHS}) + \beta_3(\text{SEX*RLHS})$	$\beta_{\text{SEX}} > 0, \beta_{\text{RLHS}} > 0, \beta_{\text{SEX*RLHS}} > 0$
Differences are due to fecal glucocorticoid levels	CORT	$\beta_0 + \beta_1(\text{CORT})$	$\beta_{\text{CORT}} < 0$
Fecal glucocorticoid level-related differences are related to reproductive life-history stage of the bird	CORT*RLHS	$\beta_0 + \beta_1(\text{CORT}) + \beta_2(\text{RLHS})$	$\beta_{\text{CORT}} < 0, \beta_{\text{RLHS}} > 0, \beta_{\text{CORT*RLHS}} > 0$
Differences are due to sampling date	DATE	$\beta_0 + \beta_1(\text{DATE})$	$\beta_{\text{DATE}} < 0$
Differences are due to sampling date in a quadratic fashion	DATE ²	$\beta_0 + \beta_1(\text{DATE}) + \beta_2(\text{DATE})^2$	$\beta_{\text{DATE}} > 0, \beta_{(\text{DATE})^2} < 0$
Differences are due to fecal glucocorticoid level and sampling date	CORT + DATE	$\beta_0 + \beta_1(\text{CORT}) + \beta_2(\text{DATE})$	$\beta_{\text{CORT}} < 0, \beta_{\text{DATE}} < 0$
Fecal glucocorticoid level-related differences are related to sampling date	CORT*DATE	$\beta_0 + \beta_1(\text{CORT}) + \beta_2(\text{DATE}) + \beta_3(\text{CORT*DATE})$	$\beta_{\text{CORT}} < 0, \beta_{\text{DATE}} < 0, \beta_{\text{CORT*DATE}} < 0$
Habitat			
Differences are due to the number of patches within an owl's territory	PATCH	$\beta_0 + \beta_1(\text{PATCH})$	$\beta_{\text{PATCH}} > 0$
Differences are due to the amount of core area within an owl's territory	CORE	$\beta_0 + \beta_1(\text{CORE})$	$\beta_{\text{CORE}} < 0$

APPENDIX B. Continued.

Hypothesis	Model	Model structure	Predicted effects ^a
Differences are due to the number of patches and the amount of core area within an owl's territory	CORE + PATCH	$\beta_0 + \beta_1(\text{CORE}) + \beta_2(\text{PATCH})$	$\beta_{\text{CORE}} > 0, \beta_{\text{PATCH}} < 0$
Differences specific to the number of patches in an owl's territory are related to the amount of core area within its territory	CORE*PATCH	$\beta_0 + \beta_1(\text{CORE}) + \beta_2(\text{PATCH}) + \beta_3(\text{CORE*PATCH})$	$\beta_{\text{CORE}} > 0, \beta_{\text{PATCH}} < 0, \beta_{\text{CORE*PATCH}} > 0$

^a Expected direction of coefficients, given that the hypothesized model is the most supported.